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(54) Title: METHOD AND AGENTS FOR PROMOTING WOUND HEALING

(57) Abstract

The present invention relates to the promotion of tissue turnover by the administration of one or more wound healing modulators. The wound healing modulator may be selected from appropriate wound healing agents and binding partners, and particularly agents that enhance wound healing. The agent may comprise advanced glycosylation endproducts (AGEs), compositions and complexes containing advanced glycosylation endproducts, derivatives thereof, and mixtures thereof. The AGEs constituting agents may particularly comprise the reaction product of a sugar selected from the group consisting of glucose, glucose-6-phosphate, fructose and ribose; and a protein selected from the group consisting of collagen, albumin, ribonuclease, avidin, and mixtures thereof. Diagnostic and therapeutic utilities are proposed, the latter including the treatment of wound healing dysfunction, the promotion of transplant tissue acceptance and the promotion of hair growth. Pharmaceutical compositions and prosthetic materials are likewise contemplated and set forth.

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METHOD AND AGENTS FOR PROMOTING WOUND HEALING

RELATED PUBLICATIONS

The Applicants are co-authors of the following articles directed to the subject matter of the present invention:
"FUNCTION OF MACROPHAGE RECEPTOR FOR NONENZYMATICALLY GLYCOSYLATED PROTEINS IS MODULATED BY INSULIN LEVELS", Vlassara, Brownlee and Cerami, DIABETES (1986), Vol. 35 Supp. 1, Page 13a; "ACCUMULATION OF DIABETIC RAT PERIPHERAL NERVE MYELIN BY MACROPHAGES INCREASES WITH THE PRESENCE OF ADVANCED GLYCOSYLATION ENDPRODUCTS", Vlassara, H., Brownlee, M., and Cerami, A. J. EXP. MED. (1984), Vol. 160, pp. 197-207; "RECOGNITION AND UPTAKE OF HUMAN DIABETIC PERIPHERAL NERVE MYELIN BY MACROPHAGES", Vlassara, H., Brownlee, M., and Cerami, A. DIABETES (1985), Vol. 34, No. 6, pp. 553-557; "HIGH-AFFINITY-RECEPTOR-MEDIATED UPTAKE AND DEGRADATION OF GLUCOSE-MODIFIED PROTEINS: A POTENTIAL MECHANISM FOR THE REMOVAL OF SENESCENT MACROMOLECULES", Vlassara H., Brownlee, M., and Cerami, A., PROC. NATL. ACAD. SCI. U.S.A. (Sept. 1985), Vol. 82, pp. 5588-5592; "NOVEL MACROPHAGE RECEPTOR FOR GLUCOSE-MODIFIED PROTEINS IS DISTINCT FROM PREVIOUSLY DESCRIBED SCAVENGER RECEPTORS", Vlassara, H., Brownlee, M., and Cerami, A. JOUR. EXP. MED. (1986), Vol. 164, pp. 1301-1309; "ROLE OF NONENZYMATIC GLYCOSYLATION IN ATHEROGENESIS", Cerami, A., Vlassara, H., and Brownlee, M., JOURNAL OF CELLULAR BIOCHEMISTRY (1986), Vol. 30, pp. 111-120; "CHARACTERIZATION OF A SOLUBILIZED CELL SURFACE BINDING PROTEIN ON MACROPHAGES SPECIFIC FOR PROTEINS MODIFIED NONENZYMATICALLY BY ADVANCED GLYCOSYLATION END PRODUCTS", Radoff, S., Vlassara, H. and Cerami, A., ARCH. BIOCHEM. BIOPHYS (1988), Vol. 263, No. 2, pp. 418-423; "ISOLATION OF A SURFACE BINDING PROTEIN SPECIFIC FOR ADVANCED GLYCOSYLATION ENDPRODUCTS FROM THE MURINE MACROPHAGE-DERIVED CELL LINE RAW 264.7", Radoff, S., Vlassara, H., and Cerami, A., DIABETES, (1990), Vol. 39, pp. 1510-1518. All of the foregoing publications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention generally relates to the repair of damaged tissues in animals and particularly humans, and, more particularly, to the modulation of the healing of wounds in such tissue.

Injury to animal tissue resulting in tissue wounds occurs from an endless variety of pathological and non-pathological causes. In response to injury, a variety of cells have been determined to cooperate to repair the damaged tissue and heal the wound. Cells resident in the local tissue participate, as do circulating blood cells specifically recruited into the wound itself and the area nearby. Dramatic changes in cellular function are required by both the resident and recruited cells in order to initiate, coordinate, and sustain the complex process of wound healing. Damaged cells and disrupted tissue matrix must be removed, new cells must be formed, and must grow and mature to replace those lost. The tissue matrix must be resynthesized and remodeled, and even the microvasculature may need to be rebuilt to supply the new tissue.

It is now recognized that cytokines exchanged among responding cells mediate the induction, control, and coordination of these and other cellular functions necessary to successfully heal the wound.

Recent interest and study of advanced glycosylation endproducts ("AGEs") has developed, and began with the observation that reducing sugars, e.g., glucose, react non-enzymatically with protein amino groups to form a diverse series of protein bound moieties with fluorescent and crosslinking properties. These compounds have been implicated in structural and functional alteration of proteins during aging and in certain diseases that are considered degradative and undesirable, e.g., the embrittlement of structural proteins observed in long-term diabetes. Several AGEs have been identified on the basis of de novo synthesis and tissue isolation procedures.

In Parent Application Serial No. 907,747 and in applications following therefrom, the use of AGEs and ther agents for the recognition and removal of in vivo

resident advanced glycosylation endproducts was disclosed. The admininstration of these agents, however, was for the essentially catabolic purpose of mobilizing the macrophage to recognize and remove AGEs. Since AGEs accumulate in pathological states, it was not apparent what role AGEs might play in the promotion and facilitation of wound healing. It is therefore to this determination and other discoveries that the present Application is directed.

SUMMARY OF THE INVENTION

In accordance with the present invention, diagnostic and therapeutic protocols are proposed that are predicated in part on the discovery that advanced glycosylation endproducts (AGEs) and other agents previously described for the promotion of the in vivo recognition and removal of AGEs, possess a definitiv modulating effect upon the progression of tissue reconstruction and wound healing in mammals, and are capable of stimulating and promoting both activities.

Accordingly, the present invention relates in its broadest aspect to the treatment of wounds as well as various wound healing dysfunctions by the administration of a wound healing modulator comprising a material selected from the group consisting of agents capable of modulating wound healing, binding partners thereto, and the muteins and fragments thereof, wherein the agents are selected from advanced glycosylation endproducts (AGEs), compositions and complexes containing advanced glycosylation endproducts alone or bound to a carrier, derivatives thereof, and mixtures thereof. The carrier may include a material selected from carbohydrates, proteins, synthetic polypeptides, lipids, bio-compatible natural and synthetic resins, antigens, and mixtures thereof. The agent could include other advanced glycosylation endproducts that may be prepared synthetically or from the reaction between sugars and other macromolecules, and monokines which stimulate phagocytic cells to increase their activity toward advanced glycosylation endproducts.

Accordingly, the agent may comprise the compounds FFI or AFGP bound to a protein such as albumin. Alternately, the agent may comprise a synthetically

derived advanced glycosylation endproduct which is prepared, for example, by the reaction of glucose or glucose-6-phosphate with albumin. This reaction product can be used alone or with a carrier in the same fashion as the FFI-albumin complex.

A monokine that functions as an agent comprises the protein known as Tumor Necrosis Factor (TNF) and its variant discovered and isolated by one of the inventors herein and named "cachectin". This material may be administered alone or in conjunction with other agents.

In addition, the agents of the present invention may be administered in conjunction with materials identified hereinafter as "co-stimulatory" agents. The coadministration of the agent with the co-stimulatory agents has been found to potentiate the activity of the former. Suitable co-stimulatory agents include monokines such as Interleukin-1 (IL-1) and gamma-interferon.

Particular therapeutic applications contemplate the administration of the wound healing modulators to specific tissues and organs to promote tissue remodelling and reconstruction. For example, AGEs useful in accordance herewith may be introduced to particular tissues by injection to stimulate and promote cellular growth or regrowth, in some instances, that will result in the structural and functional enhancement of the treated tissues.

The growth stimulating effect of the modulators of the invention finds a further application in the promotion of tissue acceptance and the corresponding limitation or inhibition of tissue rejection in tissue transplantation and reconstruction. For example, fibers implanted to promote tissue regrowth, transplanted organs or synthetic organs prepared in some instances, at least in part from structural proteinaceous material such as collagen, may have applied to them one or more of the present wound healing modulators before implantation, to promote acceptance of the exogenous material and incorporation into the target tissue. The modulators in this instance may be bound to the protein or may be otherwise adherently applied as a coating by known techniques. Accordingly, the invention extends to the corresponding

prosthetic materials having the modulators applied as a coating thereto, or chemically linked by known methods such as for example, coupling thereto a synthetic AGE such as FFI-BSA or the like.

Another application of the modulators of the present invention derives from the observations presented below, that the application of the wound healing modulator to skin stimulates hair growth. It is apparent from this that the stimulation of hair growth may be achieved by the topical or intradermal application of the present modulators, and the present invention accordingly extends to a method of stimulating hair growth thereby. This method may comprise the local application of the modulator to the dermal area, with the formulation of the modulator dosage contemplating both topical applications and if appropriate, dermal implants or injections.

The AGEs that may serve as agents may comprise the reaction product of a sugar selected from the group consisting of glucose, glucose-6-phosphate, fructose and ribose; and a protein selected from the group consisting of collagen, albumin, ribonuclease, avidin, and mixtures thereof. Particularly, the agent may comprise an advanced glycosylation endproduct selected from the group consisting of the chromophores 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI), 1-alkyl-2-formyl-3,4-diglycosyl-pyrrole (AFGP), alone and coupled to carriers such as BSA, HSA, and avidin.

Suitable agents also include materials capable of promoting agent production and/or activity, and materials capable of mimicking agent activity, such as homologous agents derived synthetically or from other cellular sources or from other species. The agent binding partners contemplated by the invention include anti-agent antibodies, receptors for the agents, materials not antibodies that antagonize the production and/or wound healing modulating activity of the agents, binding partners thereto, and other binding partners thereof. The present wound healing modulators may comprise materials that are capable of acting in vivo, and in a further embodiment, may be promoters of wound healing.

The invention accordingly includes a method for promoting wound healing comprising administering an effective amount of one of the above wound healing modulators individually, or in mixture with each other or formulated as a pharmaceutical composition. More particularly, the modulators contemplated for use in this method comprise those agents and binding partners that act as promoters of wound healing, and extend for example, to homologous agents derived from other cellular sources or from other species, materials capable of promoting agent production and/or activity, and materials capable of mimicking agent activity.

Pharmaceutical compositions may be prepared in accordance with the invention and comprise therapeutically effective amounts of the present wound healing modulators, either alone or in admixture with each other, and a pharmaceutically acceptable diluent or carrier. The modulators may preferably be present in amounts effective to deliver at least 100 ng/cm^2 and preferably from about 1 μg/cm^2 to about 10 μg/cm^2 thereof.

The therapeutic methods of the present invention apply generally to mammals and contemplate veterinary use as well as application to humans. The particular therapeutic protocols will vary accordingly upon the subject of treatment.

In the instance where wound healing may be beneficially monitored, such as to identify suspected disorders affecting wound healing, the present invention contemplates a method for measuring the activity of the wound healing modulators of the present invention. The method comprises retrieving a sample of wound fluid which such disorder is suspected, and incubating the sample with a quantity of a wound healing modulator of the present invention bearing an appropriate detectable label. The sample may thereafter be examined to determine whether such aberrant cellular activity is due to a deficiency in wound healing factor presence or activity, and to thereby attempt to isolate and identify the cause of such disorder. The present invention may also extend to appropriate new drug assays and test kits including the wound healing modulators of the present invention.

Accordingly, it is a principal object of the present invention to provide a method for treating wound healing dysfunctions in mammals.

It is a further object of the present invention to provide a method as aforesaid that is applicable to the promotion of tissue turnover and wound healing.

It is a yet further object of the present invention to provide pharmaceutical compositions for use in therapeutic methods for treating wound healing dysfunctions and/or promoting wound healing which comprise or are based upon certain wound healing modulators including agents and their binding partner(s).

It is a still further object of the present invention to provide a method for promoting wound healing by the administration of the pharmaceutical composition as aforesaid.

It is a still further object of the present invention to provide a method for promoting hair growth by the topical application of the modulators of the present invention as aforesaid.

It is a still further object of the present invention to provide a method for promoting implant acceptance and incorporation by the application to the implant material prior to implantation of the wound healing modulators as aforesaid.

It is a further object of the present invention to provide a method for measuring the activity of the wound healing modulators as aforesaid, that also serves to evaluate possible disorders in wound healing.

Other objects and advantages will become apparent to those skilled in the art from a review of the ensuing description.

DETAILED DESCRIPTION

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. Therefore, if appearing herein, the following terms shall have the definitions set out below. The term "stimulus" and its plural as used herein are intended to apply to invasive events such as infection, as well as conditions caused by wounding, and to idiopathic or spontaneous states that may for example, originate from cellular or metabolic derangements or other causes.

The terms "wound healing modulator", "agent", "AGE" and "AGEs" as used throughout the present application and claims refer to protein material having the profile of activities set forth herein and in the Claims. The terms "AGE" and "AGEs" are used as appropriate to refer to advanced glycosylation endproducts which are in the form of stable compounds and intermediates which are produced synthetically, in vivo, and in vitro by the reaction of reducing sugars with protein amino groups. AGEs therefore encompass intermediates as well as stable endproducts that are implicated in the structural and functional alteration of proteins seen during aging. For example, AGEs are recognized to react with free polypeptide amino groups, which leads to protein crosslinking. Additionally, such AGES are observed in elevated levels in circulation and in tissues in certain diseases, e.g. diabetes mellitus.

When the designations "AGE-RNAse", "AGE-Hb", "AGE-BSA", "AGE-HSA", "AGE-albumin", "AGE-collagen" and "AGE-LDL" are used, each refers to the advanced glycosylation endproducts which are formed upon chemical reaction of the substrates RNAse, Hb, BSA, HSA, albumin, collagen and LDL, respectively with a reducing sugar. Thus, AGE-RNAse refers to the advanced glycosylation endproducts of the reaction between bovine ribonuclease and a reducing sugar.

Albumin, when recited generically, refers to any species from which it was obtained, e.g., human, bovine, etc.

BSA refers to bovine serum albumin.

HSA refers to human serum albumin.

RNAse refers to ribonuclease generally, and where appropriate, to bovine pancreatic ribonuclease in particular.

Collagen is used in the conventional sense to refer to any type of collagen and derived from any appropriate source. When a specific type of collagen was used, such as in the example, the particular type is noted. However, it is recognized that alternative collagen types can also be used.

Where present, the term "FFI-BSA" refers to a model AGE-protein produced by mixing 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole hexanoic acid with bovine serum albumin and coupling the reactive compounds with dicyclohexylcarbodiimide.

Accordingly, proteins displaying substantially equivalent or altered activity are likewise contemplated. These modifications may be deliberate, for example, such as modifications obtained through site-directed mutagenesis, or may be accidental, such as those obtained through mutations in hosts that are producers of these materials. Also, the terms "wound healing modulator" and "agent" are intended where appropriate, to include within their scope proteins specifically recited herein as well as all substantially homologous analogs and allelic variations.

An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term encompasses, inter alia, polyclonal, monoclonal, and chimeric antibodies, the last mentioned described in further detail in U.S. Patent Nos. 4,816,397 and 4,816,567.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an

allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

The phrase "therapeutically effective amount" is used herein in the qualitative sense to mean an amount sufficient to promote the healing of a non-healing wound. Quantitatively, this phrase means an amount sufficient to promote, and preferably accelerate by at least about 10 percent, more preferably by at least 20 percent, a clinically significant change in the rate or extent of wound healing as a result of the administration of the wound healing modulator of the present invention.

In its primary aspect, the present invention concerns methods of diagnosing and treating wound healing dysfunction by resort to the identification and administration of certain modulators of wound healing, including certain agents represented in part by a class of compounds known as advanced glycosylation endproducts (AGEs) that are believed to take part in the promotion of wound healing.

As discussed earlier, the present invention includes therapeutic methods employing the wound healing modulators identified herein and compositions containing the same for use in such methods. Accordingly, the wound healing modulators of the present invention comprising the agents, their homologs, similarly active drugs, their receptors, their binding partner(s) or other ligands or agents exhibiting either mimicry or antagonism to the agents or control over their production, may be prepared in pharmaceutical compositions, with a suitable carrier and at a strength effective for administration by various means to a patient having a tissue wound or a wound healing disorder or dysfunction, for the treatment thereof.

A variety of administrative techniques may be utilized, among them topical applications as in ointments or on surgical and other topical appliances such as, surgical sponges, bandages, gauze pads, and the like. Also, such compositions may be administered by parenteral techniques such as subcutaneous, intravenous and intraperitoneal injections, including delivery in an irrigation fluid

used to wash body wound areas, catheterizations and the like. Average quantities of the wound healing modulator may vary and in particular should be based upon the recommendations and prescription of a qualified physician or veterinarian.

In particular, concentrations of the wound healing modulator may range from at least about 100 ng/cm^2, and preferably from about 1 μg/cm^2 to about 10 μg/cm^2 may be used. The exact quantities of the wound healing modulator administered may vary and should be based upon the recommendations and prescription of a qualified physician or veterinarian.

As mentioned earlier, the materials that function as modulators of wound healing extend to the binding partners of the agents defined herein, and particularly include the antibodies, receptors, materials not antibodies to the agents that antagonize the production and/or wound healing modulating activity of the agents and other binding partners thereto. In the instance of certain agents such as AGE-RNAse, specific antibodies that would antagonize the modulating effect that they exert on wound healing could be identified.

Antibodies, including both polyclonal and monoclonal antibodies, and drugs may also be raised to the agent and may be utilized where appropriate for the purpose of modulating wound healing by a mammalian host. In particular, the agent may be used to produce antibodies to itself in a variety of animals, by known techniques such as the hybridoma technique utilizing, for example, fused mouse spleen lymphocytes and myeloma cells. The resulting antibodies could then be prepared in a suitable pharmaceutical composition and administered to the intended host. The exact quantities, intervals of administration and administrative techniques respecting such pharmaceutical compositions may vary in accordance with those known in the medical arts, and upon the specific instruction of a qualified physician or veterinarian.

Similarly, the agents may bind to particular naturally occurring binding activities including cell associated and soluble receptors to facilitate intracellular transmission of messages relating to wound healing activity, and these binding

activities or receptor molecules may be identified as they form complexes with the agents, and thereafter may be isolated and prepared in sufficient quantities to be used in the same fashion as the agents themselves, to modulate wound healing activity. By way of illustration and not limitation, a variety of diverse receptor systems are known, such as the tyrosine kinases and G-protein receptors are already known and operate to transmit messages to the genetic material of the cell to cause corresponding changes in protein synthesis, and the present invention contemplates that these molecules and other functionally similar molecules, may participate in wound healing modulation in accordance herewith.

The present invention also relates to a variety of diagnostic applications, including methods for detecting or investigating disorders or dysfunctions in wound healing by reference to the ability of the present wound healing modulators of the present invention comprising the agents and their binding partners to promote or inhibit wound healing activity. As mentioned earlier, the agents or their binding partners could be appropriately labeled and placed in contact with a sample of wound inflammatory fluid, tissue or blood from a mammal in which the disorder is suspected. Thereafter, the sample could be examined to determine the location and status of the labeled material as well as the general activity of the sample, i.e. whether wound healing activity has increased or decreased.

As indicated earlier, the following examples set forth the details of the investigation and identification of the wound healing promoting activity of the stated inflammatory cytokines. Naturally, the specific materials and techniques set forth hereinafter are exemplary only and may vary, so that the following is presented as illustrative but not restrictive of the present invention.

EXAMPLE 1

Promotion of Wound Healing in vivo by AGEs

This series of experiments sought to determine whether advanced glycosylation endproducts such as AGE-collagen tested herein were involved in any way in

promoting wound h aling. Accordingly, AGE-collagen and unmodified collagen were assessed in a standard porcine model of wound healing in response to partial thickness skin injury.

Materials and Methods

Surgical Wounding

The wound healing model used in these studies is a modification of that described by Eaglstein and co-workers ("New Method for Assessing Wound Healing: The Effects of Triamcinolone Acetonide and Polyethylene Film Occlusion," J. of Invest. Derm., 71:382-384, 1978). Young White Yorkshire pigs weighing 10-15 kg were used in all wounding experiments. Anesthesia was induced in the following manner. Pigs received pre-operative medication with Azaperone (1 mg/lb), Atropine (0.04 mg/kg) and Ketamine (10 mg/kg) by i.m. injection. Animals were then taken to the surgical suite, intubated and maintained under Isofluorane inhalation anesthesia and nitrous oxide at 1.5 L/min. The animals were given supplemental oxygen during the procedure and maintained on a warming blanket.

Once adequate anesthesia had been attained, the back and dorsal thorax of the pigs was shaved and prepared with a 70% alcohol solution. Temperature was monitored throughout the operation, and an i.v. line (started after the animal was asleep) was maintained. Depth of anesthesia was monitored by corneal reflexes and withdrawal to painful stimuli. Anesthesia was titrated to maintain an unresponsive state. Partial thickness epidermal wounds were then made with a Padgett dermatome to a depth of 0.015 inches. Wounds were 2 x 5 cm in area. This type of injury, which removes the epidermis and a zone of the superficial dermis but spares the hair follicles, has been previously shown to be comparable to a second degree burn injury or a donor site for a skin graft.

Once all of the wounds had been created (eight pairs of wounds were made, one wound of each pair on each side of the midline), the wounds were treated and dressed. The animals were then allowed to emerge from anesthesia and

monitored closely for pain. Pain was treated with Demerol (10 mg/kg i.m.) every 4-6 hours as needed.

Treatment of Wounds

Partial thickness cutaneous wounds were treated in pairs; one wound of each pair was treated with AGE-collagen in vehicle (PBS), the other treated with an amount of collagen alone in vehicle. Wounds were treated in a pattern of bilateral pairs, whereby every AGE-collagen treated wound was matched to a contralaterally corresponding wound treated with collagen alone, at the same location on the other side of the midline of the back.

Solutions of purified AGE-collagen and collagen alone, were prepared in vehicle from concentrated stocks (below) to the desired final concentrations. A 50 μl drop of AGE-collagen- or collagen-vehicle or vehicle alone was applied to each wound, and spread over the wound with the tip of a sterile pipette.

Wounds were individually sealed with a semipermeable dressing: Benzoin was applied as an adhesive to a zone of intact skin surrounding the perimeter of each wound and the wound was sealed with a patch of Opsite applied to cover the wound and extend over and adhere to the zone of Benzoin-treated intact skin surrounding each wound. Opsite-sealed wounds were then covered with a bulky dressing.

Dosages

Wounds were treated with AGE-collagen/collagen at the time of wounding and daily thereafter, at various dosages from 1 to 10 μg AGE-collagen or unmodified collagen per 50 μl vehicle (PBS) per wound. Because wounds measured approximately 2 x 5 cm, this dosage corresponds to 0.1 to 1 μg AGE-collagen or unmodified collagen per cm^2 wound area, respectively.

Biopsy Technique and Histological Analysis

Biopsies of healing wounds were initially taken on days 3, 4 and 5 after wounding. Preliminary analysis of control wounds revealed that day 4 wounds were best suited t display differences in the rate or degree of healing, and day

4 biopsies were obtained from subsequent tests. Control wounds on day 4 typically show mild residual inflammation and fibroblast activity with persisting ulceration in the sense that epidermal regrowth is still incomplete and portions of the dermis remain exposed. Where epidermis is reforming from the margins of the wound and focally from the hair follicles spared by wounding, there is good granular layer formation with areas of overlying cornified epithelium and little parakeratosis. At this incompletely healed stage, then, a variety of histological characteristics of wound healing are intermediate.

Acceleration or retardation of wound healing is manifest to a trained dermatopathologist by light microscopic comparison of histological sections prepared from biopsies of the healing wounds. Histological sections from wounds in which healing has been accelerated by treatment can be expected to have less remaining ulceration or even complete recoverage by epidermis with good granular layer formation and a more complete overlying cornified layer, and little or no residual fibroblast proliferation or parakeratosis. These histological criteria for completeness of healing can be expected to vary in the other direction if wound healing is retarded by treatment.

To assess the rate and completeness of healing, test pigs were anesthetized as at wounding, wound dressings were removed, and the wounds photographed for gross characterization of healing. When fully anesthetized, test pigs were then overdosed with SleepAway euthanasia solution. Elliptical biopsy samples through the full skin thickness (into the layer of subcutaneous fat) and extending across the full width of the wound and into intact skin on either side were then cut from each wound by hand using a scalpel. Biopsy samples were individually fixed by immersion in 10% buffered formalin in coded containers which did not reveal what treatment the parent wound had received. Coded samples were then routinely processed for light microscopic histological analysis by sectioning at 5 μm, mounting on slides, and staining with hematoxylin and eosin. The biopsies were cut into histological sections in a plane normal (perpendicular) to the surface of the skin, so as to include both the full extent of the wound and a small margin of non-wounded skin at each end of the section.

The degree of wound healing was assessed by measuring the linear extent of re-epithelialization across the full width of the histological sections from the wound biopsy; that is, from the boundary of non-wounded skin on one side of the section to the boundary of non-wounded skin at the other side of the wound. Measurements were taken microscopically, by using a calibrated ocular reticule to measure the linear extent of the total wound, and the linear extent of re-epithelialized wound. The linear extent of re-epithelialized wound was then expressed as a percentage of the linear extent of the total wound, and this percentage was taken as a measurement of the degree of wound healing.

RESULTS

The sections taken 3 days after healing show a marked difference between tissue treated with AGE-collagen and that treated with unmodified collagen. The treated tissue shows completely healed epidermis, with full epithelialization overlying a slightly cellular dermis. The epidermis shows focally a good granular layer, and only minimal parakeratosis. The control tissue, while fully epithelialized, still contains many fibroblasts within the dermis, and there is only poor granular layer formation. This suggests a much less advanced healing wound. Similar changes are seen in tissue taken 4 and 5 days post wounding. The tissue treated with AGE-collagen consistently shows more mature appearing epidermis, with full granular and minimal overlying parakeratosis. The control sections, while almost completely epithelialized, still show poor granular layer formation and extensive overlying parakeratosis.

EXAMPLE 2

In this series of experiments, the healing properties of silk sutures reacted with sugar in order to produce AGEs were investigated. The following protocol was followed.

Rat Study Design:

Male Lewis rats (350-450 g) were made diabetic by intravenous injection of streptozotocin (65 mg/kg). One week later, they were bled, and their plasma

tested for glucose levels. Rats with glucose levels greater than 400 mg/dl were considered diabetic. Diabetic and non-diabetic rats were then divided into groups:

TABLE A

	Unbrowned sutures	Sutures browned with ribose
Normal Controls	5	5
Diabetics	5	5

Two lots of silk sutures (Davis and Geck, Danbury, CT) were tested; untreated sutures and AGE-modified sutures. AGE-modified sutures were prepared by incubating the sutures in 200 mM ribose in 100 mM phosphate buffer, pH 7.4 for 2 weeks. The sutures were rinsed in PBS prior to use. There were 5 normal control rats for each suture lot, and 5 diabetic rats for each lot, for a total of 20 rats. (See Table A, below)

For implantation of sutures, the rats were anesthetized using a mixture of Xylazine and Ketamine (1:9), given intramuscularly at a level of .001 mg/g body weight. The backs of the rats were shaved, washed with soap and water, sprayed with alcohol, and swabbed with Provadine. An incision of approximately 5 cm was made in the skin along the midline of the back. A subcutaneous continuous stitch was used to close the incision, using the appropriate suture lot. The incision sites were visually inspected on a daily basis to monitor healing. The animals were sacrificed at 14 days using CO_2. The skin from their backs was removed, and evaluated visually as to how they have healed in comparison with the control groups.

The extent of wound closure was evaluated, blinded to group, by three individuals and graded on a scale of 1+ to 3+ with 1+ being the least healed and 3+ being the best closure with little or no scar. The degree of vascularization was evaluated by examination of the underlying skin using a light box. This enabled the investigator to visualize by transmitted light the amount of blood vessels formed along the suture line. Vascularity was evaluated on a 1+ to 4+ scale with 1+ being little vascularity around the incision site and 4+

being the greatest degree of vascularity. All animals were coded so that the final evaluations were done blinded as to the treatment group.

TABLE B

WOUND HEALING PROPERTIES OF AGE TREATED SUTURES
OBSERVATIONS AT SACRIFICE

Group I: Diabetic Animals/Control sutures					Body Weights	
Rat #	Closure	Mean Score*	Vascular-ization	Mean Score	Start (9/16)	Finish (10/1)
A	1+	1.40	1+	1.40	337	318
B	2+		1+		373	359
C	1+		2+		385	384
D	1+		2+		325	304
E	2+		1+		290	292
Group II: Diabetic Animals/AGE-sutures						
Rat #	Closure	Mean Score	Vascular-ization	Mean Score		
F.	2+	2.40	2+	1.80	321	332
G	3+		1+		333	322
H	1+		1+		285	273
I	3+		2+		274	282
J	2+		1+		323	301
P	1+		2+		337	324
Group III: Normal Animals/Control sutures						
Rat #	Closure	Mean Score	Vascular-ization	Mean Score		
K	---------- Died ----------					
L	2+	2.25	2+	2.25	337	335
M	2+		2+		350	353
N	2+		2+		402	411
O	3+		3+		366	379
Group IV: Normal Animals/AGE-sutures						
Rat #	Closure	Mean Score	Vascular-ization	Mean Score		
Q	3+	2.60	3+	2.80	400	415
R	3+		3+		408	419
S	2+		1+		406	397
T	2+		4+		390	396
U	3+		3+		381	387

* Mean score is the sum of the individual animal scores divided by the number of animals

Closure
1+ = not healed well
2+ = healed well
3+ = healed very well; little or no scar

Vascularity
1+ = little or no vascularity around incision site
2+ = some vascularity around incision site
3+ = moderate vascularity around incision site
4+ = large amounts of vascularity around incision site

. Rat # F had some pus oozing from incision

TABLE C

SUMMARY OF WOUND HEALING DATA:

Animal Group	Sutures Used	% of Rats in Each Category: Closures 1+	Closures 2+	Closures 3+	Vascularization 1+	Vascularization 2+	Vascularization 3+	Vascularization 4+
I - Diabetic	Control	60	40	0	60	40	0	0
II - Diabetic	AGE-suture	33	33	33	50	50	0	0
III - Normal	Control silk	0	75	25	0	75	25	0
IV - Normal	AGE-suture	0	40	60	20	0	60	20

EXAMPLE 3

Mouse Study Design

25 non-diabetic male C_3H/Hej mice (25-30g) were anesthetized as described above. Their backs were shaved, washed with soap and water, sprayed with alcohol, and swabbed with Provadine. An incision of approximately 2-2.5 cm was made in the skin along the midline of the back. A cutaneous interrupted stitch was used to close the incision, using either control silk sutures or AGE-silk sutures (incubated with ribose as described in Example 2). The animals were sacrificed 7 days later and the skin removed as described above. The closure could not be evaluated adequately in mice due to the thin nature of the skin and the dark color of the strain being tested. Therefore, only the degree of vascularity was assessed as described above. One obvious but unexpected occurrence in this study was related to the degree of new hair growth in mice whose wounds were closed using AGE sutures. These results are included in Table D, below.

TABLE D

WOUND HEALING PROPERTIES OF AGE SUTURES IN MICE

Animal Group	Sutures	Number of Mice in Each Category (% of Total) Vascularization**				Number of Mice Showing An Increase in Hair Growth on Back
		1+	2+	3+	4+	
Normal	Control Sutures (n=15)	5 (33.3%)	5 (33.3%)	3 (20%)	2 (13.3%)	1 (6.6%)
Normal	AGE-Sutures (n=10)	0 (0%)	2 (20%)	4 (40%)	4 (40%)	7 (70%)

** See Table B for evaluation code

As indicated by the above data, the application of AGE to the skin in this fashion resulted in the promotion of hair growth. While the data shown is preliminary in nature, it clearly demonstrates this activity, and by virtue of its presence in an animal model that is considered credibly predictive of behavior in higher species, lends support to the application of the wound healing agents of the invention to this cosmetically constructive activity.

As indicated earlier, the agents herein may be formulated for the treatment of a variety of mammals, including domestic and farm animals, as well as humans, to control the wound healing process, and where desired, to assist in its promotion and the promotion of tissue growth and reconstruction as described and as evidenced hereinabove.

This invention may be embodied in other forms or carried out in other ways without departing from the spirit or essential characteristics thereof. The present disclosure is therefore to be considered as in all respects illustrative and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A method of treating wound healing dysfunction and/or promoting wound healing in mammals including humans, comprising administering to a mammal a therapeutically effective amount of a wound healing modulator comprising a material selected from the group consisting of an agent for enhancing wound healing, binding partners thereto, and muteins and fragments thereof, wherein said agent is selected from the group consisting of advanced glycosylation endproducts, compositions and complexes containing advanced glycosylation endproducts, derivatives thereof, and mixtures thereof.

2. The method of Claim 1 wherein said agent is bound to a carrier.

3. The method of Claim 2 wherein said carrier is selected from the group consisting of carbohydrates, proteins, synthetic polypeptides, lipids, bio-compatible natural and synthetic resins, antigens, and mixtures thereof.

4. The method of Claim 1 wherein said agent comprises the reaction product of a sugar selected from the group consisting of glucose, glucose-6-phosphate, fructose, ribose, and mixtures thereof; and a protein selected from the group consisting of collagen, albumin, ribonuclease, avidin, and mixtures thereof.

5. The method of Claim 1 wherein said agent comprises an advanced glycosylation endproduct selected from the group consisting of FFI and AFGP alone; FFI and AFGP coupled to a carrier selected from BSA, HSA, and avidin; and mixtures thereof.

6. The method of Claim 1 wherein said wound healing modulator is selected from the group consisting of said agent, homologous agents derived from other cellular sources, homologous agents derived from other species, materials capable of promoting agent production and/or activity, materials capable of mimicking agent activity, muteins and fragments thereof, and mixtures thereof.

7. The method of Claim 1 wherein said binding partners to said agent are selected from the group consisting of anti-agent antibodies, a receptor for the agent, a material not antibody to the agent that antagonizes the production and/or wound healing modulating activity of the agent, and mixtures thereof.

8. The method of Claim 1 wherein said agent is derived from cells which are produced by recombinant DNA technologies.

9. The method of Claim 1 wherein said agent is administered in a concentration of at least about 100 ng/cm^2 of wound area.

10. The method of Claim 1 wherein said agent is administered in a concentration of from about 1 μg to about 10 μg/cm^2 of wound area.

11. A pharmaceutical composition for the treatment of wound healing dysfunction and/or for promoting wound healing in mammals, including humans, comprising:

A. a pharmaceutically effective amount of a wound healing modulator comprising a material selected from the group consisting of an agent for modulating wound healing, homologous agents derived from other cellular sources, homologous agents derived from other species, a material capable of promoting agent production and/or activity, a material capable of mimicking agent activity, binding partners thereto, and muteins and fragments thereof, wherein said agent is selected from the group consisting of advanced glycosylation endproducts, compositions and complexes containing advanced glycosylation endproducts, derivatives thereof, and mixtures thereof; and

B. a pharmaceutically acceptable carrier.

12. The composition of Claim 11 wherein said agent comprises the reaction product of a sugar selected from the group consisting of glucose, glucose-6-phosphate, fructose, ribose, and mixtures thereof; and a protein selected from the group consisting of collagen, albumin, ribonuclease, avidin, and mixtures thereof.

13. The composition of Claim 11 wherein said agent comprises an advanced glycosylation endproduct selected from the group consisting of FFI and AFGP alone; FFI and AFGP coupled to a carrier selected from BSA, HSA, and avidin; and mixtures thereof.

14. The composition of Claim 11 wherein said agent is present in an amount of at least about 100 ng dose.

15. The composition of Claim 11 wherein said agent is present in an amount of from about 1 μg/dose to about 10 μg/dose.

16. The composition of Claim 11 wherein said agent is derived from cells which are produced by genetic replication.

17. An antibody to an agent for modulating wound healing, wherein the agent to which said antibody is raised comprising is selected from the group consisting of advanced glycosylation endproducts, compositions and complexes containing advanced glycosylation endproducts, derivatives thereof, and mixtures thereof.

18. The antibody of Claim 17 wherein said agent comprises the reaction product of a sugar selected from the group consisting of glucose, glucose-6-phosphate, fructose, ribose, and mixtures thereof; and a protein selected from the group consisting of collagen, albumin, ribonuclease, avidin, and mixtures thereof.

19. The antibody of Claim 17 wherein said agent comprises an advanced glycosylation endproduct selected from the group consisting of FFI and AFGP alone; FFI and AFGP coupled to a carrier selected from BSA, HSA, and avidin; and mixtures thereof.

20. The antibody of Claims 17, 18 or 19 wherein said agent is derived from cells which are produced by genetic replication.

21. A method for detecting a disorder in wound healing in a mammal comprising measuring the activity of a wound healing modulator selected from the group consisting of an agent for modulating wound healing, homologous agents derived from other cellular sources, homologous agents derived from other species, a material capable of promoting agent production and/or activity, a material capable of mimicking agent activity, an anti-agent antibody, a receptor for the agent, a material not antibody to the agent that antagonizes the production and/or wound healing modulating activity of the agent, binding partners thereto, and muteins and fragments thereof, wherein said agent is selected from the group consisting of advanced glycosylation endproducts, compositions and complexes containing advanced glycosylation endproducts, derivatives thereof, and mixtures thereof, said method for detecting comprising:

A. preparing at least one sample of said agent;

B. placing a detectable label on said agent sample;

C. placing the labeled agent sample in contact with a tissue sample from a wound from said mammal in which disorder is suspected; and

D. examining said tissue sample to locate said labeled material, and measuring to determine the activity of said agent.

22. The method of Claim 21 wherein said agent is bound to a carrier.

23. The method of Claim 22 wherein said carrier is selected from the group consisting of carbohydrates, proteins, synthetic polypeptides, lipids, bio-compatible natural and synthetic resins, antigens, and mixtures thereof.

24. The method of Claim 21 wherein said agent comprises the reaction product of a sugar selected from the group consisting of glucose, glucose-6-phosphate, fructose, ribose, and mixtures thereof; and a protein selected from the group consisting of collagen, albumin, ribonuclease, avidin, and mixtures thereof.

25. The method of Claim 21 wherein said agent comprises an advanced glycosylation endproduct selected from the group consisting of FFI and AFGP

alone; FFI and AFGP coupled to a carrier selected from BSA, HSA, and avidin; and mixtures thereof.

26. The method of Claims 21-24 or 25 wherein said agent is derived from cells which are produced by recombinant DNA technology.

27. A method for stimulating hair growth on a particular area of the body surface of a mammal comprising administering to said area an effective amount of a wound healing modulator, said wound healing modulator comprising a material selected from the group consisting of an agent for enhancing wound healing, binding partners thereto, and muteins and fragments thereof, wherein said agent is selected from the group consisting of advanced glycosylation endproducts, compositions and complexes containing advanced glycosylation endproducts, derivatives thereof, and mixtures thereof.

28. The method of Claim 27 wherein said agent is bound to a carrier.

29. The method of Claim 28 wherein said carrier is selected from the group consisting of carbohydrates, proteins, synthetic polypeptides, lipids, bio-compatible natural and synthetic resins, antigens, and mixtures thereof.

30. The method of Claim 27 wherein said agent comprises the reaction product of a sugar selected from the group consisting of glucose, glucose-6-phosphate, fructose, ribose, and mixtures thereof; and a protein selected from the group consisting of collagen, albumin, ribonuclease, avidin, and mixtures thereof.

31. The method of Claim 27 wherein said agent comprises an advanced glycosylation endproduct selected from the group consisting of FFI and AFGP alone; FFI and AFGP coupled to a carrier selected from BSA, HSA, and avidin; and mixtures thereof.

32. A method for promoting host acceptance and incorporation of transplanted tissue and suture material comprising applying to said tissue and suture material an effective amount of a wound healing modulator, said wound healing modulator comprising a material selected from the group consisting of an agent for enhancing wound healing, binding partners thereto, and muteins and fragments thereof, wherein said agent is selected from the group consisting of advanced glycosylation endproducts, compositions and complexes containing advanced glycosylation endproducts, derivatives thereof, and mixtures thereof.

33. The method of Claim 32 wherein said agent is bound to a carrier.

34. The method of Claim 33 wherein said carrier is selected from the group consisting of carbohydrates, proteins, synthetic polypeptides, lipids, bio-compatible natural and synthetic resins, antigens, and mixtures thereof.

35. The method of Claim 32 wherein said agent comprises the reaction product of a sugar selected from the group consisting of glucose, glucose-6-phosphate, fructose, ribose, and mixtures thereof; and a protein selected from the group consisting of collagen, albumin, ribonuclease, avidin, and mixtures thereof.

36. The method of Claim 32 wherein said agent comprises an advanced glycosylation endproduct selected from the group consisting of FFI and AFGP alone; FFI and AFGP coupled to a carrier selected from BSA, HSA, and avidin; and mixtures thereof.

37. A prosthetic implant comprising a biocompatible material selected from organ tissue and synthetic suture material, said biocompatible material having applied thereto an effective amount of a wound healing modulator, said wound healing modulator comprising a material selected from the group consisting of an agent for enhancing wound healing, binding partners thereto, and muteins and fragments thereof, wherein said agent is selected from the group consisting of advanced glycosylation endproducts, compositions and complexes containing advanced glycosylation endproducts, derivatives thereof, and mixtures thereof.

38. The prosthetic implant of Claim 37 wherein said agent is bound to a carrier.

39. The prosthetic implant of Claim 38 wherein said carrier is selected from the group consisting of carbohydrates, proteins, synthetic polypeptides, lipids, bio-compatible natural and synthetic resins, antigens, and mixtures thereof.

40. The prosthetic implant of Claim 37 wherein said agent comprises the reaction product of a sugar selected from the group consisting of glucose, glucose-6-phosphate, fructose, ribose, and mixtures thereof; and a protein selected from the group consisting of collagen, albumin, ribonuclease, avidin, and mixtures thereof.

41. The prosthetic implant of Claim 37 wherein said agent comprises an advanced glycosylation endproduct selected from the group consisting of FFI and AFGP alone; FFI and AFGP coupled to a carrier selected from BSA, HSA, and avidin; and mixtures thereof.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/01677

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02; C07K15/00; G01N33/68; A61F2/10

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
E	WO,A,9 304 086 (THE ROCKFELLER UNIVERSITY) 4 March 1993 see page 3, line 33 - page 14, line 2	7
X	EP,A,0 259 893 (THE ROCKFELLER UNIVERSITY) 16 March 1988 cited in the application see column 9, line 36 - column 14, line 4 see column 19, line 30 - column 27, line 8	1-6, 11-13, 21-25
A	WO,A,9 000 060 (COLLAGEN CORPORATION) 11 January 1990	
A	EP,A,0 157 359 (NATIONAL JEWISH HOSPITAL AND RESEARCH CENTER) 9 October 1985	

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 JUNE 1993	13. 07. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	REMPP G.L.E.

Form PCT/ISA/210 (second sheet) (January 1985)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 93/01677

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-10 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort jusufying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9301677 SA 71118

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 29/06/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		US-A-	5202424	13-04-93
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EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82